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DEVELOPMENT OF A SYNTHETIC POLYMER BURN COVERING.(U)

JUL 77 J B GREGORY, J D GRESSER, D L WISE N00014-73-C-0201

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DYNATECH R/D COMPANY

Development of a Synthetic
Polymer Burn Covering

Progress Report on Contract N00014-73-C-0201
Period: January 1, 1977 to June 30, 1977
Dynatech Report No. 1631

Sponsored by the Office of Naval Research
Contract Authority N00014-73-C-0201
Task No. NR 207-044/12-02-76(444)

Prepared by:

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J.D. Gresser
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Prepared for:

Director, Medicine and Dentistry Programs
Biological and Medical Sciences Division
Office of Naval Research
Department of the Navy
800 North Quincy Street
Arlington, Virginia 22217

Date Submitted:

July 18, 1977

a division of DYNATECH CORPORATION

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During the period January 1, 1977, to June 30, 1977, a new plasticized version of the poly-ε-caprolactone (PCL) wipe-on solution designed for use as an immediate post-burn treatment has been evaluated at NMRI on pigs under the supervision of Captain Burgoon D.V.M. Initial results show a significant improvement in the rate of burn healing and reduction of scar formation.

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Work on the measurement of insensible water loss by resistance hygrometry and development of suitable packaging for the wipe-on coating will be continued during the balance of the year.

The up-take of labelled proline has been demonstrated to correlate with the formation of new collagen in burned areas using rats. The technique does not distinguish differences between the rates of healing of burns covered by the wipe-on coating and uncoated control burns.

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Using ^{14}C labelled PCL, it has been demonstrated that PCL is not absorbed by the tissues from the wipe-on coating. In addition to the work to develop a wipe-on coating, development of a flexible burn covering has continued. A new, cut plush knit PCL fabric laminated to a plasticized PCL film has been demonstrated to be better than any previous combination tested in conforming to complex and changing shapes such as the back of a rat. More similar fabric is being knit for further testing by Dynatech and the Navy. The practicability of measuring by autoradiography the ingrowth of new tissue into the fabric film laminate applied over full excision wounds has been demonstrated. Autoradiography experiments are to be carried out later in 1977.

Sterilization studies of both the new wipe-on coating and the new fabric film laminate will be conducted in 1977.

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Section 1

SUMMARY

During the period January 1, 1977 to June 30, 1977, a new plasticized version of the poly-ε-caprolactone (PCL) wipe-on solution previously designed for use as an immediate post-burn treatment has been evaluated at NMRI on pigs under the supervision of Captain Burgoon D.V.M. Initial results show a significant improvement in the rate of burn healing and reduction of scar formation.

Work on the measurement of insensible water loss by resistance hygrometry and development of a suitable packaging for the wipe-on coating will be continued during the balance of the year.

The up-take of labelled proline has been demonstrated to correlate with the formation of new collagen in burned areas using rats. The technique does not distinguish differences between the rates of healing of burns covered by the wipe-on coating and uncoated control burns.

Using ^{14}C labelled PCL, it has been demonstrated that PCL is not absorbed by the tissues from the wipe-on coating. In addition to the work to develop a wipe-on coating, development of a flexible burn covering has continued. A new, cut plush knit PCL fabric laminated to a plasticized PCL film has been demonstrated to be better than any previous combination tested in conforming to complex and changing shapes such as the back of a rat. More similar fabric is being knit for further testing by Dynatech and the Navy. The practicability of measuring by autoradiography the ingrowth of new tissue into the fabric film laminate applied over full excision wounds has been

demonstrated. Autoradiography experiments are planned to be carried out later in 1977.

Sterilization studies of both the new wipe-on coating and the new fabric film laminate will be conducted in 1977.

Section 2

INTRODUCTION

The program at the Dynatech R/D Company to develop a synthetic polymer burn covering is midway through the fifth year. The main objective of the program is to develop a synthetic polymer burn covering that will reduce dehydration, sepsis, sodium loss, scar formation, morbidity, and mortality in burn patients. The material is to be producible at low-cost and in large quantities so to be immediately available in event of large scale disasters.

The specific tasks proposed for the fifth year are the following.

Task 1 - Continued Testing and Evaluation of Immediate Post-Burn Wipe-on Treatment System

Close communications and assistance with the pig testing at NMRI will be an integral part of the program. Techniques will be used to quantify the results on animal testing such as resistance hygrometry, use of ^{14}C labelled proline to measure quantitatively the rate of wound healing and of ^{14}C-poly-ε-caprolactone for evaluationof possible polymer up-take. A practical applicator for wipe-on fluid will also be developed.

Task 2 - Complete Preparation and Evaluation of Poly-ε-caprolactone Fabric/Laminate Burn Covering

Work on this task will continue, but at a substantially reduced level. Special attention will be given to the completion of all polymer, yarn, and fabric preparation during this present contract year. Evaluation of

selected fabric/laminates on animals will be carried out, including adherence measurements and determination of ^{14}C proline ingrowth into the fabric.

Task 3 - Supply Samples and Coordinate Work with NMRI

The Contractor shall maintain close coordination between work at NMRI on swine and the burn covering testing and evaluation at Dynatech. Samples will be supplied to NMRI and results using this material on smaller animals and other tests will be well documented.

Task 4 - Evaluation of Burn Covering Materials in Anticipation of Clinical Trials

Standard sterilization procedures will be evaluated and tested on both the wipe-on and the fabric/laminate burn treatment systems. Radiation sterilization and ethylene oxide sterilization will be evaluated. Appropriate FDA staff will be contacted to determine potential requirements. Communications with FDA staff on other biomaterials development programs have proved to be valuable in anticipating regulatory requirements.

This technical report covers the progress during the period January 1, 1977 through June 30, 1977, on the above tasks. The work on the wipe-on treatment system is covered in Section 3; that on the poly-ε-caprolactone (PCL) fabric-PCL film laminates is covered in Section 4. The preliminary findings of the first tests by NMRI of the wipe-on system on swine are also presented in Section 3.

The study of sterilization procedures, Task 4 above, has been postponed until later this year when the specific products likely to be used for clinical trials will be more precisely defined.

Section 3

DEVELOPMENT OF WIPE-ON SOLUTIONS FOR IMMEDIATE POST-BURN TREATMENT

3.1 Introduction

In the Fourth Annual Report on this project submitted January 31, 1977 data are given showing that one coat of a wipe-on coating containing 10% by weight of poly-ε-caprolactone (PCL) dissolved in a 4/1 by volume mixture of acetone and methylene chloride significantly reduced insensible water loss (IWL) from burned and abraded areas on the backs of rats. The coating also appeared to promote healing especially of abraded areas. Further study of PCL coatings are currently being carried out on pigs at the NMRI under the supervision of Captain Burgoon D.V.M. (USAF). During the past six months, considerable progress has been made in the study of the formulation and testing of PCL wipe-on coatings.

3.2 Evaluation of PCL Wipe-On Burn Coverings by NMRI

Dynatech engineers visited Captain Burgoon D.V.M. on June 27, 1977 to observe the tests of our wipe-on coating on young pigs burned with a 2.22" diameter branding iron heated to 65^{o}C in an oil bath. Each pig, which is 10 to 12 weeks old, is shaved on both sides. The iron is applied in four places on each side with dwell times of 10, 15, 25, and 45 seconds respectively. A freshly heated iron is used for each application. The burns on the left side of the pig serve as controls. Those on the right side are covered by brushing with one coat of wipe-on coating using a nylon paint brush. The burns will be observed for healing and photographed weekly for a period of at least two months. The 10 second burn is 2nd degree and heals naturally. The 15 second

burns are borderline between 3rd degree and 2nd. The 25 and 45 second burns are 3rd degree and heal only by formation of granulation tissue. A circle is tatooed around each burn to delineate the area. Changes in the area of the tatooed circle during the healing process are indicative of scar tissue formation.

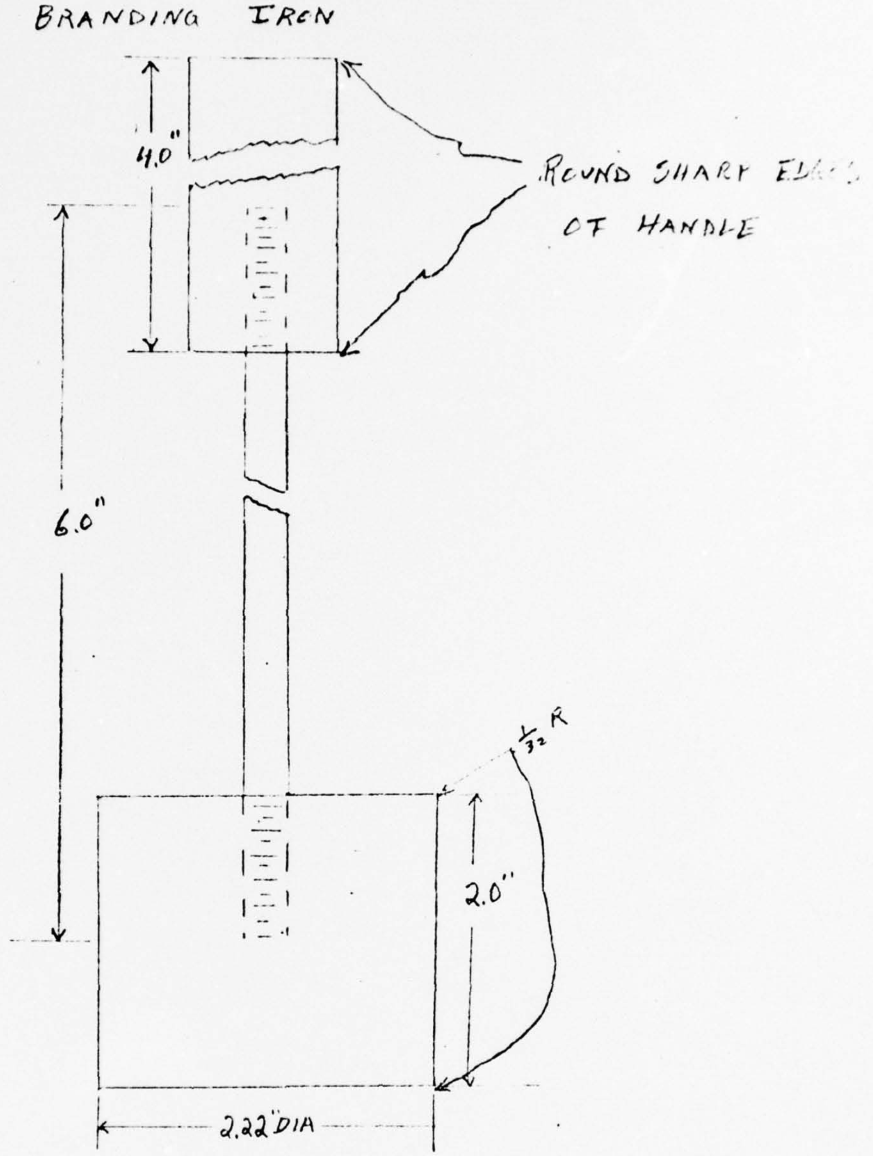
Formulation 26359 containing no plasticizer peeled off rather easily and was only marginally effective. Formulation 26387 containing plasticizer was more adherent and significantly effective in promoting healing, and reducing scar formation. The formulations submitted are as follows:

	26359 Submitted 01/26/77	26387 Submitted 05/23/77
Poly-ε-Caprolactone	100g	100g
Triethyl Citrate	---	33g
Methylene Chloride	200ml	300ml
Acetone	800ml	---
Methyl Acetate	---	1200ml

3.3 Measurement of Skin Contact Temperature (TC.)

The Dynatech R/D Company manufacturers a thermesthesiometer (Trade name Thermotouch) used for measuring the temperature which skin reaches when in contact with various surfaces. The instrument is constructed according to the specifications and procedure given in National Bureau of Standards Technical Note 816. The skin contact temperature, or TC value, varies with the nature of the surface coming in contact with the skin as well as with its temperature and contact time. For example, a metal surface feels much hotter and is more hazardous than a plastic or wood surface having the same temperature. One of these instruments was used to determine the dependence of the TC value of an iron similar to the one used to brand the pigs (See Figure 1) upon 1)

Figure 3.1



MATERIALS

1" DIA MAPLE DOWEL 4" LONG SIZE O DIA DRILL 1" DEEP IN CENTER OF END $\frac{3}{8}$-24 TAP

$\frac{3}{8}$" DIA DRILL ROD 6" LONG $\frac{3}{8}$-24 THREAD 1" FROM EACH END

2.22 DIA STAINLESS STEEL IRON 2" LONG SIZE O DIA DRILL IN CENTER OF 1 END 1.0" DEEP $\frac{3}{8}$-24 TAP

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whether the iron is heated in air or water and 2) upon whether the iron is wet or dry.

The results given in Table 3.1 show the Tc value to be the same whether the iron is heated to a given temperature in an oven or in a water bath and whether it is wet or dry. We were unable to test irons heated in an oil bath such as that used at NMRI as any oil on the iron would damage the probe on the Thermotouch instrument. However, if the oil is wiped off the iron carefully, the Tc value would not be expected to be affected by this mode of heating. Comparison of the results presented in Table 3.1 with the data plotted shows that with the iron at 67°C, at least an 8 second contact time is required to product a third degree burn on typical human skin. At NMRI, they have found that a 15 second contact time on the young pigs gives a burn which is borderline between 2nd and 3rd degree when the iron is 65°C. Figure 3.2 is taken from the NBS Technical Note 816 referenced earlier.

3.4 Measuring Pervaporation by Resistance Hygrometry

As indicated in the Fourth Annual Report on this project, a resistance hygrometer has been rented from Hygrodynamics Products, a division of American Instrument Company, Silver Springs, Maryland. We are currently using this equipment with the cup described in Section 3.6 of that report to measure the rate of water vapor permeation through the various films used to seal the vapometer cup containing water and comparing these results with those obtained by measuring water vapor permeation by weight loss. When consistent results have been obtained, the equipment will be used to measure the effectiveness of wipe-on coatings to reduce the water loss from traumatized areas on rats.

3.5 Proline Uptake by Traumatized Areas

The purpose of this experiment using rats was to determine if

Table 3.1

Tc Values Obtained from Branding Iron for Various Contact Times When the Iron is Heated in an Oven and in a Hot Water Bath[1]

Mode of Heating Branding Iron	Temperature of Branding Iron°C	Tc Temperature °C		
		2Sec. Exposure	4Sec. Exposure	8Sec. Exposur
Oven	67.2 ± 0.1	55.8 ± 0.5	58.3 ± 0.5	60.1 ± 0.4
Water Bath (Iron dried before testing)	67.6 ± 0.3	55.3 ± 0.1	57.6 ± 0.5	60.4 ± 0.3[2]
Water Bath (Iron left wet)	67.5 ± 0.4	55.6	57.7	60.5

NOTES

1. See Figure 3.1 for sketch of branding iron. Values are given ± the standard deviation. If no ± given after value only one determination was made. The iron temperatures were measured 9, 8, and 3 times respectively for each mode. The Tc values were obtained with the Thermotouch after calibration. All values except those with no ± were obtained from the mean of three readings except where indicated.

2. Average of two readings.

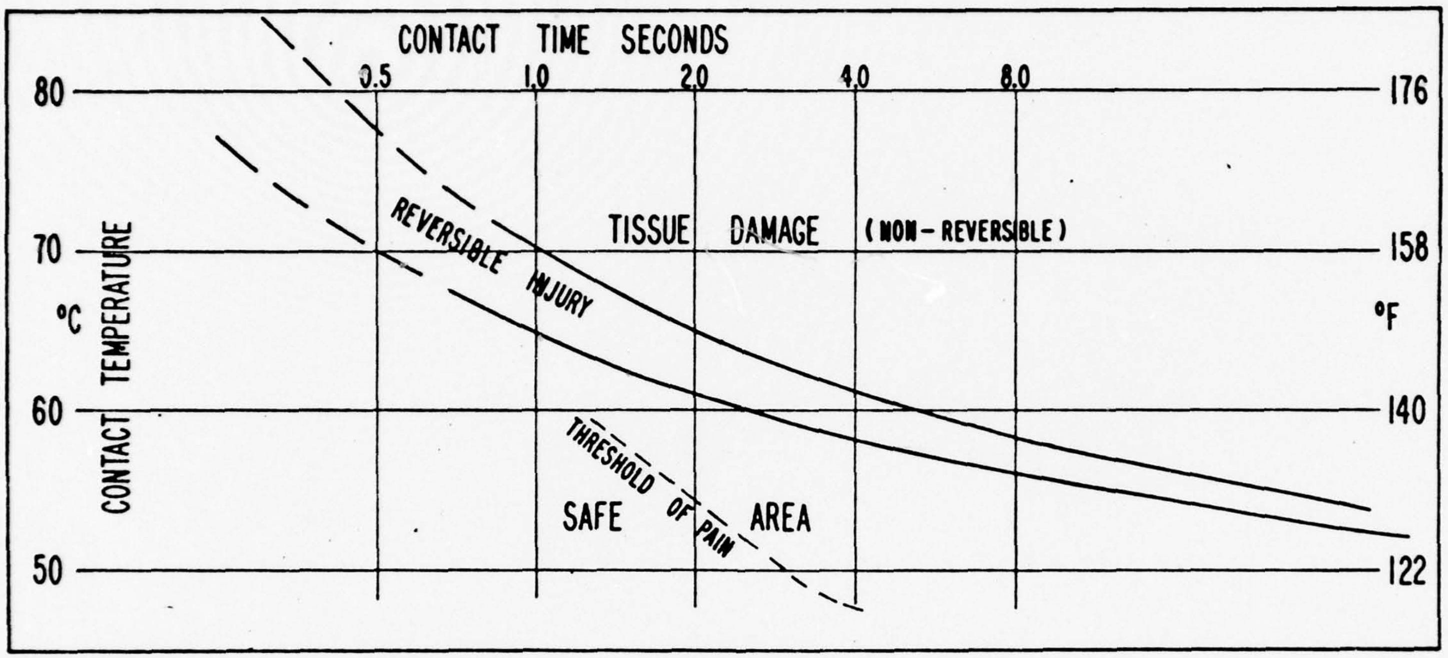


Fig. 3.2 Physiological Response Curve

there was an enhanced uptake of tritiated proline by traumatized areas and if application of a wipe-on burn covering would show reduced trauma signified by reduced proline uptake. A similar experiment was reported in Section 3.5 of the Fourth Annual Report on this project. In the latest experiments, changes in procedure were made to correct inadequacies in the previous experimental design as follows: The rats were sacrificed 24 hours after traumatization and treatment with labelled proline. The route of labelled proline administration was <u>per</u> <u>os</u> rather than I.P. Two groups of rats received a mild burn so that the degree of uptake of labelled proline by living and dead tissue (eschar) could be compared.

From the data which are summarized in Table 3.2, we conclude as follows:

1. Proline uptake by Groups II, III, and VI are almost the same and significantly greater than the controls. In these three cases, the trauma was sufficiently severe to kill the skin of that region.

2. Groups IV and V received milder trauma which did not kill the burned skin. In these cases significantly greater proline uptake was observed than for the other groups.

3. Apparently tritiated proline can be used successfully as a measure of collagen formation in traumatized areas. However, if dead eschar is formed, no proline is incorporated into this, so results on a DPM/gram basis do not reflect the high counts which would probably be obtained for newly formed living tissue.

4. Groups II and III showed similar uptake, as did groups IV and V.

We conclude from the above that while the presence of tritiated proline is

Table 3.2

Proline Uptake by Traumatized Rats (1)

Group	Treatment	Comment	DPM/g
I	No trauma (proline control)	proline control	10492.1 4426.5 4783.5 Mean (2) 6,600±3400
II	Burned with 0.50ml ethanol. Covered with 1 mil PCL (2)	Burned skin is dead	88,636.4 61,167.8 67,133.7 Mean (2) 72,300±14400
III	Burned with 0.50 ml ethanol. No Burn Cover applied. (2)	Burned skin is dead	61,655.5 80,138.1 65,753.4 Mean (2) 69,200±9700
IV	Burned with 0.25 ml ethanol. Covered with 1 ml PCL (2)	Skin not dead	58,543.7 114,599.0 137,940.0 Mean (2) 103,700±40,800
V	Burned with 0.25 ml ethanol. No Burn Covering applied (2)	Skin not dead	85,396.8 138,891.8 89,741.0 Mean (2) 104,700±29,700
VI	Cut skin, separate from peritoneum, and reseal with wound clips	Skin on cut area is dead	76,354.1 64,417.5 71,142.9 Mean (2) 70,600±6000

NOTES

1. Groups of three rats each (140-160g) were anesthetized with Penthrane after which they were shaved, depilated with Nair cream, washed, and traumatized as indicated in the above table. In some cases PCL wipe-on burn covering was applied to burned areas, but this preparation contained no radioactivity. Immediately after traumatization each animal received 1.0 ml of 10 μCi/ml aqueous L-[4-^{3}H(N)]-proline per os. Twenty four hours later animals were sacrificed and a sample of skin from the traumatized area was excised for combustion and liquid scintillation counting of ^3H_2O.

2. 20 cm^2 template used to confine alcohol and delineate burned area.

indicative of collagen formation in traumatized areas, any difference in the rate of new collagen formation between traumatized areas covered with the wipe-on coating and such areas when not covered is too small to be detected by this technique.

3.6 Absorption of PCL Through Traumatized Areas On Rats Backs When Applied Topically As a Wipe-On Coating

In this experiment, a solution of ^{14}C labelled PCL was applied to burned areas on rats backs. Forty-eight hours later the rats were sacrificed and samples of various tissues analyzed for radioactivity. Table 3.3 summarizes the results.

Note that no radioactivity was found in the blood, muscle, kidney or urine of any animal. Some activity was found in the dorsal skin of most animals. This skin was sampled from directly beneath the wipe-on covering. However, two animals out of eight had no significant activity in this skin area. It seems likely that not all of the wipe-on coating was removed prior to testing the skin and what is measured is residual wipe-on coating rather than absorbed PCL. Activity was also found in the feces of all rats except the controls and in the liver of all but one. The rats were not restrained prior to testing and since they often nibble at the wound area, they probably ingested some of the wipe-on covering.

In order to determine more accurately the origin of the fecal liver, and skin radioactivity, the experiment was repeated with the rats held in restraining cases. Results are presented in Table 3.4.

No activity was observed in the livers of any animal in this experiment. Furthermore, fecal activity was much less than previously observed indicating that although the cages do reduce the ability of the rats to nibble at the wound coverings, they were not completely

Table 3.3

Absorption of PCL Wipe-On Through Traumatized Skin, First Experiment (1)

Group	No. Rats	Treatment	Thickness of PCL Coat	Absorbed 14.C Radioactivity, DPM/gram: Blood	Kidney	Muscle	Urine	Feces	Liver	Skin
I (2)	1	No burn	No coat	None	None	None	None	None	None	None
II	2	No burn	2 mil (3)	None	None	None	None	19830.2 2909.1 Mean 11400	787.7 99.4 Mean 440	220.9 2.0 Mean 100
III	2	Burned with 0.25 ml ethanol	2 mil	None	None	None	None	38811.2 4776.3 Mean 21800	626.2 60.5 Mean 340	2236.8 0 Mean 1100
IV	2	Burned with 0.50 ml ethanol	2 mil	None	None	None	None	1724.8 949.6 Mean 1300	49.4 0 Mean 20	329.7 293.5 Mean 300
V	2	Burned with 1.0 ml ethanol	2 mil	None	None	None	None	14784.9 3709.2 Mean 9200	1145.4 107.9 Mean 630	3558.0 2315.4 Mean 2900

Notes: 1) A sample of PCL wipe-on burn covering, 10% PCL by weight, was prepared containing ^{14}C labeled PCL with a specific activity of 2.788μCi/g. Fifty grams of the PCL (Sample 26329) was dissolved in 400ml of acetone and 100ml CH_2Cl_2 to give a solution with a specific activity of 0.286μCi/gm.

Three groups of two rats each (140-160g) were anesthetized with Penthrane, their backs shaved and depilated with Nair, and burned with ethanol over an area of 11.4cm^2 (diameter of burned area = 1.5 inches).

Two other groups served as controls as indicated in the accompanying table. Forty eight hours after burning, the animals were sacrificed and samples of various tissues were analyzed for radioactivity. Tissue samples were oxidized in a Harvey Biological oxidizer and ^{14}C measured as $^{14}CO_2$ by liquid scintillation counting.

2) Group 1 served as control for background DPM.

3) Film easier to remove than in burned groups.

TABLE 3.4

ABSORPTION OF PCL WIPE-ON THROUGH
TRAUMATIZED SKIN, SECOND EXPERIMENT (1)

Absorbed ^{14}C Radioactivity, DPM/g

Tissue	Group I	Group II	Group III
Blood	-----	----(3)	-----
Feces	-----	41.3	743.7
Kidney	-----	----	-----
Liver	-----	----	-----
Muscle	-----	----	-----
Skin	-----	111.2	166.4
Urine	-----	----(4)	-----

NOTES:

1. Group I 2 control rats; unburned and uncovered
   Group II 3 rats; unburned but covered with PCL wipe-on (2)
   Group III 3 rats; burned and covered with PCL wipe-on (2)

   Group I controls were used to measure background for calculation of activities of Groups II and III. In order to minimize injestion of PCL covering all rats were placed in small wire mesh restraining cages for the 48 hours prior to sacrifice. This reduced their mobility but did not render them completely immobile; although no biting of the backs was observed, it is reasonable to assume that this did occur

2. See Table 3.3 Note 1 for formulation of PCL solution used: PCL activity = 2.700 mCi/g; solution activity - 0.286 mCi/g
3. One animal had minimal activity above background. The remaining two had none.
4. One animal had 131.1 DPM/ml; the remaining two had none. This animal was not the one with activity in the blood.

effective and that a small amount of film was ingested. The reduction in skin activity compared to the values in Table 3.3 was due to exercise of greater care in removal of the film prior to sampling the skin directly beneath it. However, it was apparently not possible to remove the wipe-on coating entirely and some ^{14}C activity was still found.

One animal in Group II had some activity in its blood, minimally above background. Another in this group had 13.11 DPM/ml above background in the urine, again a small amount. As the first experiment showed no blood or urinary activity, we are confident that this represents contamination of the sample rather than a reproducible experiment and result.

It was concluded that the PCL in the wipe-on coating is not absorbed into tissues through either normal or burn traumatized skin.

3.7 Study of Plasticized PCL Films

Initial data on plasticized PCL films was seen in Section 3.4 of the Fourth Annual Report. This work has been continued and the results of the current and past work are summarized in Table 3.5.

For the latest PCL wipe-on coating submitted for trial by Captian Burgoon (See Section 3.2 of this report), a formulation containing 33 PHR of triethyl citrate was selected as PCL films containing this amount of triethyl citrate appear to have ample strength for a wipe-on coating with a minimum increase in water vapor transmission compared to films of unplasticized PCL.

Similar films were used in making the film-fabric laminates discussed in Section 4 of this report.

To duplicate the water vapor transmission (WVT) of full thickness

TABLE 3.5

STUDY OF PLASTICIZED FILMS

PHR Plasticizer by weight (1)	PCL Polymer (1)	Nature of Plasticizer (1)	Tensile Strength (3) psi	Elongation at Yield (3) %	Young's Modulus (3) psi X 10^{-4}	WVT (4) g mm/24 hrs m^2
0	A	-	1120	2	7.5	4.62
4.5	A	A	1600 $\pm$ 600	4.2 $\pm$ 2	5.6 $\pm$ 1.1	----
4.5	A	B	1820 $\pm$ 540	4.2 $\pm$ 1.2	5.2 $\pm$ 2.3	----
9	A	A	1940 $\pm$ 650	6.3 $\pm$ 2.3	4.7 $\pm$ 1.1	----
9	A	B	1700 $\pm$ 690	5.5 $\pm$ 2.0	4.3 $\pm$ 1.7	----
18	A	A	1690 $\pm$ 170	10.3 $\pm$ 1.4	3.0 $\pm$ 0.1	5.06
18	A	B	1860 $\pm$ 340	7.8 $\pm$ 2.0	3.6 $\pm$ 0.4	6.62
25	A	B	1342 $\pm$ 183	3.4 $\pm$ 0.3	4.6 $\pm$ 6.8	7.6 $\pm$ 0.3
33	(5)	B	853 $\pm$ 107	3.8 $\pm$ 0.5	2.8 $\pm$ 0.9	8.5 $\pm$ 0.5
50	B	B	554 $\pm$ 120	3.5 $\pm$ 0.2	1.4 $\pm$ 0.5	28.2 $\pm$ 0.2

NOTES:

(1) A basic solution was made up containing 50 grams of PCL polymer 22392, 100 mls of methylene chloride and 400 mls of acetone giving a 10% by weight solution of PCL. PHR = Parts by weight per hundred parts of resin. Two different polymers were using polymer A, 22392 molecular weight 135K and polymer 26311 modular weight 152K.

(2) The amounts of plasticizer to give the parts by weight indicated of plasticizer based on 100 parts of polymer were added to the base solution. A = triacetin (Eastman Kodak Co.); B = triethyl citrate (Charles Pfiser and Co. Citroflex 2).

(3) Films of the solution were cast on release paper using a film caster set at 0.025 inches and allowed to air dry and then evacuated for 24 hrs. giving a dry film thickness of about 1 mil. Dumbell samples were cut using Die C specified in ASTM Method D412 and tested for tensile and elongation at yield and Young's modulus using an Instron Tensile Tester Model TTC with a cross-head speed of 0.1 inches per minute.

(4) Water vapor transmission obtained at 37°C and 0% relative humidity using ASTM Method E96 Procedure D.

(5) Duplicate determinations made,one set using polymer A and the second using polymer B. See note (1).

normal human skin which is about 83g mm/24 hr.-m^2 (See Note), a PCL film having a WVT of 8.5g.-mm/24 hr.-m^2 should be about 0.1 mm thick (0.004"). The thickness of a one coat application of the wipe-on solutions being tested by Captain Burgoon (See Section 3.2) is 0.02 to 0.04 mm (1 to 2 mils) but since the eschar over the traumatized area is also a moisture barrier even though not as good as normal skin, the water loss from a burned area covered by the wipe-on coating, should be close to that of normal skin. We will be able to obtain actual values on rats to verify this assumption when the evapometer mentioned in the previous section has been calibrated and can be used to measure the IWL from normal and traumatized skin both with and without a wipe-on coating.

3.8 Solvents for PCL Wipe-On Solutions

The first PCL wipe-on solution tested was made by dissolving PCL in tetrahydrofuran. Aromatic solvents are not suitable for this application because of their toxicity, so an attempt was made to formulate the wipe-on solution using non-aromatic solvents. A number of solvent combinations were tested using their solubility parameters as a guide but no satisfactory combinations of low boiling oxygenated straight chain hydrocarbons were found. However, both acetone and methyl acetate are satisfactory solvents if mixed 4/1 by volume with methylene chloride. The 4/1 methyl acetate/methylene chloride is the better solvent of the two and is the solvent used in the plasticized PCL wipe-on coating being tested by Captain Burgoon. See Table 3.6 for data.

Captain Burgoon stated that for field application, he would like to have the wipe-on coating supplied in a tin can with a brush as an integral part of the cover rather than in an aerosol can. He stated that there was a danger that an aerosol will rupture at the temperature which can be reached under field conditions.

(Note: See Table 4.1 in the First Annual Report for data on the water vapor transmission of human skin.)

TABLE 3.6

STUDY OF SOLVENTS FOR POLY-ε-CAPROLACTONE

No.	Solvent	Volume Ratio of Solvents in Mixture	Solubility Parameter (1) λ	λd	λa	Boiling Pt. °C	Solvent for PCL
1	Benzene	--	9.15	9.03	1.48	79	yes
2	Dioxane	--	9.74	8.55	4.65	101	yes
3	Methylene chloride	--	9.53	8.52	5.08	46	yes
4.	Tetrahydrofuran	--	9.52	9.25	2.28	78	yes
5.	Acetone	--	9.10	7.49	5.19	--	no
6.	Methyl Acetate	--	9.49	7.56	5.72	--	no
7.	Ethyl Acetate	--	9.10	7.44	5.19	--	no
8.	Methyl Ethyl Ketone	--	9.27	7.77	5.06	--	no
9.	Methanol	--	14.28	7.42	12.40	--	no
10.	Ethanol	--	12.92	7.73	10.45	--	no
11.	Ethyl Ether	--	7.62	7.05	2.88	--	no
12.	Acetone, Ethanol	9/1	10.1	7.62	6.5	--	Fair, not equal to number 15
13.	Methyl Ethyl Ketone Ethanol	82/18	9.92	7.74	6.04	--	no
14.	Methyl Ethyl Ketone Ethanol	75/25	10.18	7.75	6.41	--	no
15.	Acetone Methylene Chloride	4/1	9.29	7.65	5.18	--	Yes, gels at about 15°C
16.	Acetone Methylene Chloride	3/1	9.29	7.65	5.18	--	Better solvent than 15
17.	Acetone Ethanol	95/5	9.90	7.59	6.35	--	Fair not equal to number 15
18.	Acetone Ethanol	90/10	10.1	7.62	6.5	--	delete
19.	Methyl Ethyl Ketone Ethanol	82/18	9.92	7.74	6.04	--	delete
20.	Methyl Ethyl Ketone Ethenol	75/25	10.18	7.75	6.41	--	poor
21.	Ethyl Ether Ethanol	56/44	9.95	7.34	2.90	--	no
22.	Ethyl Acetone Ethanol	78/22	9.94	7.46	6.40	--	no
23.	Methyl Acetate Ethanol	9/1	10.17	7.60	6.24	--	Fair not equal to number 5
24.	Methyal Acetate Methanol	9/1	10.45	7.53	6.63	--	Fair not equal to number 5
25.	Methyal Acetate Methylene Chloride	4/1	9.58	7.75	5.60	--	Gels at a lower temperature than number 15, hence, best solution of mixtures tested

NOTES

(1) For solubility parameters for individual solvents see C. M. Hansen J. of Paint Tech 39, 104 (1967). Values for mixtures calculated by assuming such component contributes to the total in proportion to the volume fraction present.

3.9 Status of Proposed Program and Plans for the Remainder of the Calendar Year

This section covers Tasks 1 and 3 of the program for 1977 described in Section 2.

Dynatech personnel are cooperating with NMRI with the testing of the PCL wipe-on coating on pigs and initial results with the plasticized wipe-on coating show significant improvements in wound healing. Work is continuing on the use of resistance hygrometry for measuring insensible water loss (IWL) over burned areas and animal tests with the apparatus developed are scheduled for July or August. Uptake of labelled proline has been demonstrated to correlate with the formation of new collagen in burned skin areas using rats, but any difference in the rate of collagen formation between traumatized areas covered with wipe-on and those not were too small to permit measurement of the improvement by this technique.

Use of ^{14}C labelled PCL has demonstrated that there is no significance of PCL uptake by the tissues from the wipe-on coating when it is applied to burns on the backs of rats.

The wipe-on is currently being supplied in cone top cans and a separate nylon brush is used for application of the coating. Captain Burgoon is of the opinion that a similar package, except having the brush an integral part of the can cover, would be quite suitable for field use. This package would be preferable to use of an aerosol can which could rupture at the high temperatures sometimes encountered in the field. Work is continuing on development of an aerosol package which would be suitable for hospital use.

Cooperation on the pig testing at NMRI will continue during the balance of 1977 as will development of suitable wipe-on packaging.

Section 4

SYNTHETIC GRAFT

4.1 Introduction

The Fourth Annual Report on this project reports that two fabrics knit at Titone, Inc. in Burlington, NJ, under the direction of Professor Thomas Edman of the Philadelphia College of Textiles and Science would be evaluated during the coming year. One of the fabrics is a looped velour similar to fabric IV-22 described in Table 4.2 of the Fourth Annual Report and the second is a cut plush fabric with an open weave designed to conform well when spread over a complex shape such as a rat's back or a knee or elbow. The cut plush should be easier to remove from the healing tissue than the looped velour evaluated previously since the pile of a cut plush is formed by individual fibers rather than loops.

4.2 Preparation and Evaluation of PCL Fabric-PCL Film Laminates

Laminates were prepared by bonding 25 μ thick films of PCL plasticized with 33 PHR of triethylcitrate to the back of the fabrics. Four different laminates were evaluated as follows:

1. Laminate using looped velour similar to fabric IV-22 (See Table 4.2 of the Fourth Annual Report on this project).

2. Laminate using cut plush with a pile height of 0.8 mm (1/8").

3. Ditto with a pile height of 1.6 mm (1/16").

4. Same as 3 except sample cut on the bias.

The detailed results of the tests on the laminates when applied to full excision wounds on rats' backs and the test procedure are given in Table 4.1a and 4.1b. We found that the cut plush fabric laminates conformed better to the rat's back than the looped velour laminates and that there was slightly less damage to the underlying tissue when the pile of the cut plush was 0.8 mm high rather than 1.6 mm. Applying laminates cut on the bias helped the graft to conform to the complex curves of the rat's back. Use of plasticized PCL in place of the unplasticized PCL used in earlier tests also helped the fabric to conform easily to the undulations in the rat's back.

It should be noted from the data obtained that the average laminate adhesion to the rat's back was about the same on day 1 as on days 3 and 6. However, we noted that failure of the new tissue was pronounced when the fabric was removed the next day, but by day 6 tissue failure was minimal. Apparently, it takes several days for the new tissue growing into the PCL fabric to become strong enough to withstand having the fabric stripped from the wound without damage.

Samples of the tissue from the underlying wound were taken from the backs of rats 19 and 20 and preserved in formalin so that they could be examined under the microscope to determine if there was any difference in the kind of tissue between the whitish non-vasculated tissue obtained from the back of rat 19 and the red, bloody, well vasculated tissue obtained from the back of rat 20. The microscopic examination showed that both samples are typical healthy granulation tissue with no significant difference between them.

Figures 4.1 to 4.4 illustrate some of the results obtained on rats with the cut plush fabric. Figures 4.1 and 4.2 show the excellent adhesion due to growth of new tissue into the fabric one day after application. Figure 4.1 shows tissue failure on rat No. 8. Figure 4.2 shows good adhesion but no tissue failure on rat No. 38. Figure 4.3 shows how well the new fabric conforms to the back of the rat. Figure 4.4 illustrates how the PCL fabric PCL film laminate can be removed 6 days after application with minimal tissue tearing.

Table 4.1a

ADHESION TO FULL THICKNESS EXCISION WOUNDS OF

PCL FABRIC - PCL FILM LAMINATES (1)

Rat No.	Laminate No.	Fabric No. (2)	PCL Film Thickness μ	Adhesion g/cm after (3) 1 day	3 days	6 days	Comments
1	26379A	26347-1	38	75			Stiff fibers in one small area of fabric.
2	26379B	"	48	70			
3	26379C	"	48		109		Tissue failure.
4	26379D	"	38		111		Tissue failure.
5	26379E	"	38			78	Good conformance - lot of blood in new tissue. No tissue failure.
6	26379F	"	38			54	Little or no vascularization in new tissue. Good conformance. Two small pockets - no infection visible.
		Average	41	73	110	66	
7	26384A	26347-2A	25	54			Tissue failure.
8	"	"	"	124			Tissue failure.
9	"	"	"	50			
10	"	"	"	43			Tissue failure.
11	"	"	"	14 (5)			Fabric dry - little adhesion.
12	"	"	"		64		Tissue failure.
13	"	"	"		43		Fabric conformed well to rat's back.
14	"	"	"		18 (5)		Fabric conforming to rat's back. One small pocket near edge.
15	"	"	"		54		Fabric conforming. One small pocket.
16	"	"	"		59		Like 15 except some tissue failure near shoulder of rat.
17	"	"	"			rat died	
18	"	"	"			59	One small infected pocket. Some tissue failure.
19	"	"	"			45	New tissue not vasculated. Part of new tissue carried away on burn covering part. No tissue tearing below surface layer
20	"	"	"			63	New tissue well vasculated. No tissue failure.
21	"	"	"			64	
		Average	25	68	55	58	

NOTES

(1) See Table 4.1b for concluding data as well as for explanation of notes.

Table 4.1b

ADHESION TO FULL THICKNESS EXCISION WOUNDS OF

PCL FABRIC - PCL FILM LAMINATES(1)

Rat No.	Laminate No.	Fabric No.(2)	PCL Film Thickness μ	Adhesion g/cm after(3) 1 day	3 days	6 days	Comments
22	26384B-1	36347-2B	25	50			Poor bond one side.
23	-2	"	"	34			Poor bond one side.
24	-3	"	"	23			
25	-4	"	"	41			Small unbonded area.
26	-5	"	"	45			Small unbonded area.
27	-6	"	"		45		Some tissue failure one side. Rest strips well
28	-7	"	"		21		Good fit. Strips well at sides. Some tissue failure over rat's spine.
29	-8	"	"		36		Good fit. Strips well.
30	-9	"	"		84		One wrinkle. Considerable tissue failure.
31	-10	"	"		88		One small pocket. Some tissue failure.
32	-11	"	"			50	Covering strips cleanly from tissue
33	-12	"	"			64	One large pocket. No apparent infection.
34	-13	"	"			54	Well vasculated new tissue. Burn covering strips clean.
35	-14	"	"			43	Same as 34.
36	-15	"	"			45	Some weakly adherent areas with little new tissue beneath.
		Average	25	39	65	51	
37	26384C-1	36347-2A(4)	"	47			Tissue failure.
38	-2	"	"	25			*Small unbonded area.*
39	-3	"	"	25			
40	-4	"	"		43		Tissue failure.
41	-5	"	"		27		Good fit. Strips well.
42	-6	"	"		21		Good fit. Strips well.
43	-7	"	"			39	Well vasculated new tissue.
44	-8	"	"			52	Some tissue failure. One small pocket - infected.
		Average	25	32	30	46	

NOTES

(1) Films of PCL were cast from a solution using a Bradley Blade set to give the desire dry film thickness onto a sheet of plate glass treated with Frekote 33 (Frekote, Inc.) polished with a paper towel wet with THF. The solution contained 100 parts by wt. of PCL batch 26341 (mol. wt. 152,000) plus 33 parts by wt. of triethyl citrate (Citroflex 2 Chas. Pfizer & Co., Inc.) dissolved in 888 parts by wt. of THF. 4 minutes after casting, the fabric was pressed against the tacky films. After 2 hours, the laminate was removed and evacuated overnight at room temperature to remove the residual THF. After measuring the film thickness, the laminates were trimmed to 5 x 8 cm and the corners rounded to a radius of ~ 2.5 cm. The samples were applied to full excision wounds on the backs of rats (approx. wt. 150 g) using 12 evenly-spaced stainless steel wound clips. At the time indicate, the animals were injected I.P. with a lethal dose of pentobarbitol, the wound clips were removed, the fabric detached from the skin, trimmed to a width of 4 cm, the rat mounted on a harness and the fabric pulled from the rat using an Instron Tensile Tester at a rate of 1.2 cm/min (jaw separation rate 2.4 cm/min) in a posterior-anterior direction.

(2) Description of fabrics: 26347-1 - PCL brushed velour with Dacron backing similar to fabric IV22 (see Table 4.2 and page 3.7 in 4th annual report dated Jan. 31, 1977, Dynatech Report #1571). 26347-A - PCL cut plush with pile cut to 1.6 mm. Dacron backing (see page 37 in 4th annual report op.cit.) 26347-2B - same as above except pile 0.8 mm high.

(3) Average adhesion in lbs/in obtained by measuring the area under stress strain curve plotted by the x,y, recorder of the Instron Tensile Tester and dividing by the product of the length of the curve in the strain direction and the average sample width in inches. To convert g/cm to lbs/in divide by 179. Laminates applied on 3/29/77.

(4) *Fabric samples cut on bias.*

(5) Not included in average.

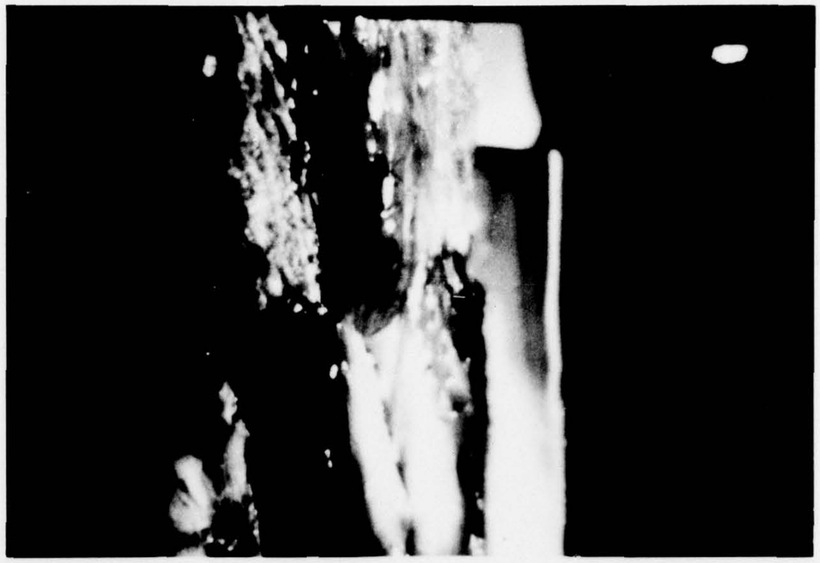


Figure 4.1 Rat Number 8 sacrificed one day post application of laminate. Note that the tissue has grown into the fabric well but is too weak to withstand removal without tearing.

Figure 4.2 Rat Number 38. Sacrificed one day post application of laminate. Note good adhesion as in Figure 4.1, but that this time tissue did not tear when fabric was removed.

Figure 4.3 Rat three days after application of laminate. Note excellent conformance of laminate to the rat's curved back.

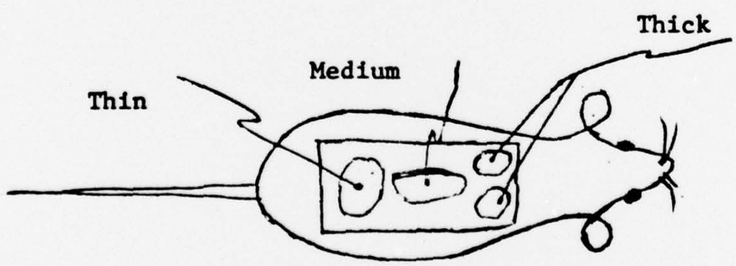
Figure 4.4 Rat 19 sacrificed six days post application of laminate. Note that fabric can be removed without tearing the underlying tissue and that underlying tissue is strong and healthy.

1500 g of PCL fiber prepared for us last year by the Southern Research Institute has been sent to Professor Edman for preparation of additional cut plush fabric with a pile height of 0.8 mm (1/8"). We expect to obtain enough material for the additional tests planned by Dynatech in 1977 and to have enough left to permit limited trials by the Navy.

4.3 Tissue Growth into PCL Fabric-PCL Film Laminate Burn Coverings as Measured by Uptake of Tritiated Proline

The purpose of this experiment was to determine from the up-take of tritiated proline the rate at which tissue from the traumatized area grows beneath and into the burn covering. Using the data obtained, we plan to select the time, amount and specific activity of the proline injection required for future autoradiography experiments.

The samples were taken by carefully excising the fabric keeping the underlying tissue intact. The thickness of tissue adhering to the fabric varied depending upon the section of the rat's back in contact with the fabric. The following diagram illustrates this.



The raw data is summarized in table 4.2. In table 4.3 the data has been averaged according to tissue distribution. There seems to be no significant variation in the samples taken from the rats given all the proline in one injection and sampled 24 hours later and those given two injections at 24 hour intervals and sampled 48 hours after the first injection. Since only two rats were used for each group, large variations between individuals may mask trends.

TABLE 4.2

Up-Take of Tritiated Proline in Tissue beneath PCL Fabric-PCL Film Burn Coverings.

Group	Sample	Tissue Distribution	DPM/Gram	DPM/cm^2
I	26347-2B-1	Thin	5,222,504	509,557
	-2	Medium	1,997,578	260,557
	-3	Thick	2,209,448	437,016
	-2D-1	Thin	1,284,596	61,363
	-2	Medium	2,095,147	185,570
	-3	Thick	2,595,290	418,259
II	26347-2I-1	Thin	5,618,613	402,082
	-2	Medium	1,529,459	317,727
	-3	Thick	550,460	99,083
	-2E-1	Thin	3,367,150	309,375
	-2	Medium	5,137,096	1,720,927
	-3	Thick	689,165	205,291
III	26347-2L-1	Thin	2,458,559	546,773
	-2	Medium	2,071,287	496,270
	-3	Thick	2,072,084	1,092,679
	26347-2S-1	Thin	2,743,363	363,630
	-2	Medium	2,816,087	713,049
	-3	Thick	2,823,769	897,331

Table 4.3

Up-Take of Tritiated Proline in Tissue Beneath PCL Fabric-PCL Film laminate Burn Coverings.

Summary of Data from Table 4.2

Group	Treatment	Tissue Distribution	Tritium Recovery: $DPM \times 10^{-3}$/gram of fabric plus Underlying Tissue	Tritium Recovery: $DPM \times 10^{-3}/cm^2$ of fabric plus plus underlying tissue
I	one 5μCi/g dose Tissue sampled 24hrs later	Thin	3254 ± 1969	285 ± 224
		Medium	2046 ± 49	223 ± 38
		Thick	2402 ± 193	428 ± 9
		Mean (1)	2567 ± 1369	312 ± 172
II	Two 2.5μ Ci/g doses at 24hr intervals-Tissue sampled 48hrs after 1st.	Thin	4493 ± 1126	356 ± 46
		Medium	3333 ± 1804	1019 ± 702
		Thick	620 ± 69	152 ± 53
		Mean (1)	2815 ± 2230	509 ± 603
III	Two 1.25μ Ci/g doses as in group II	Thin	2601 ± 142	455 ± 92
		Medium	2444 ± 372	605 ± 108
		Thick	2448 ± 376	995 ± 98
		Mean (1)	2498 ± 356	685 ± 272

Notes: (1) Mean of all values from Table 4.2 ± standard deviation.

The experiments show clearly, however, that there is enough tritium in the tissue of all groups for future autoradiography experiments. We therefore conclude that one injection i.p. of 5μCi/g will be given and that samples will be taken for autoradiography 24 hours later.

Twenty four hours after the last injection, the fabric was removed with the underlying tissue intact. This new tissue was not evenly distributed but ranged from very thin to thick. Therefore three samples of known weight and area were taken from each burn covering for tritium analysis. These three were representative of tissue distribution, one thin to almost bare, one with a medium tissue layer, one with a thick tissue layer. Each sample was oxidized in a Harvey Biological Oxidizer, the tritiated water collected and measured by liquid scintillation counting. Table 4.2 presents results as DPM/gram as well as DPM/cm^2 for each rat.

4.4 Progress on Tasks 2 and 4 of Program Proposed for 1977 and Work Planned for the Balance of the Year

The cut plush PCL fabric which was developed in 1976 laminated to plasticized PCL film has been tested on rats and has proved to be a significant improvement in the ability to conform to the complex and changing shapes of a rat's back in comparison to all fabric film laminates tested heretofore. Additional quantities of a similar fabric are now being knit using PCL fiber spun for us during 1976 at the Southern Research Institute. We soon expect to have enough fabric for additional animal testing by Dynatech and limited testing by the Navy.

Experiments with tritium labelled proline have demonstrated that enough tritium will be absorbed in the new tissue growing into the fabric film laminate to permit study of the growth rates by autoradiography techniques. Autoradiography experiments with the new lot of fabric are planned as soon as the fabric arrives.

Sterilization procedures for both the new PCL fabric PCL film laminate and the latest plasticized wipe-on coating are planned for later this year. See Task 4 in Section 2, the introduction to this report.